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Eutrophication and warming-driven green tides (*Ulva rigida*) are predicted to increase under future climate change scenarios

Guang Gao [a,1], Anthony S. Clare [a], Craig Rose [b], Gary S. Caldwell [a,*]

[a] School of Marine Science and Technology, Ridley Building, Newcastle University, Newcastle upon Tyne NE1 7RU, England, UK
[b] Seaweed & Co. Ltd., Office 2.3 North Tyneside Business Centre, 54a Saville Street, North Tyneside NE30 1NT, England, UK

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ABSTRACT

The incidence and severity of extraordinary macroalgae blooms (green tides) are increasing. Here, climate change (ocean warming and acidification) impacts on life history and biochemical responses of a causative green tide species, *Ulva rigida*, were investigated under combinations of pH (7.95, 7.55, corresponding to lower and higher pCO_2), temperature (14, 18 °C) and nitrate availability (6 and 150 μmol L^{-1}). The higher temperature accelerated the onset and magnitude of gamete settlement. Any two factor combination promoted germination and accelerated growth in young plants. The higher temperature increased reproduction, which increased further in combination with elevated pCO_2 or nitrate. Reproductive success was highest (64.4 ± 5.1%) when the upper limits of all three variables were combined. Biochemically, more protein and lipid but less carbohydrate were synthesized under higher temperature and nitrate conditions. These results suggest that climate change may cause more severe green tides, particularly when eutrophication cannot be effectively controlled.

1. Introduction

Due largely to fossil fuels burning and land use change, the atmospheric concentration of carbon dioxide (CO_2) has increased by over 40% since 1750 and currently exceeds 400 ppm, a rate of increase unprecedented within the last 800,000 years (Gattuso et al., 2015). These emissions are driving global climate change, particularly by increasing mean global temperatures and reducing the pH of seawater. More than 90% of the thermal energy accumulated between 1971 and 2010 was absorbed by the oceans, with surface waters (upper 75 m) warming at the greatest rate (increasing between 0.09 and 0.13 °C per decade over the period 1971 to 2010; IPCC, 2013). The global mean sea surface temperatures for the months of February and August are projected to increase by 1.9 °C by the end of the 21st century. The maximum warming of around 4 °C is predicted for high latitudes of the northern hemisphere in summer (Bartsch et al., 2012).

Aside from thermal storage, the oceans are also major sinks for CO_2. When CO_2 dissolves in seawater it forms carbonic acid which decreases pH – a process termed ocean acidification. The mean surface ocean pH has already decreased by 0.1 units since the beginning of the industrial era, corresponding to a 26% increase in hydrogen ion concentration (IPCC, 2013). It is predicted that by 2100 the average surface ocean pH may decrease by 0.5 units below pre-industrial levels if CO_2 emissions continue at current trajectories (Raven et al., 2005). Seawater at high latitudes is expected to experience more serious acidification since more CO_2 can dissolve in cold waters compared to tropical regions (McNeil and Matear, 2008; Roleda et al., 2012). Coastal waters are also more susceptible to acidification than the pelagic ocean due to eutrophication processes as bacterial respiration of algae biomass further depresses seawater pH (Cai et al., 2011).

Anthropogenic eutrophication driven by increased urbanization and use of the coastal zone, as well as rising fertilizer use, has led to accelerated nutrient inputs to coastal waters (Carpenter et al., 1998; Smith et al., 1999). Eutrophication poses a growing threat for many coastal ecosystems (Bricker et al., 2008). One consequence of eutrophication is the promotion of green tide events – extraordinary blooms of macroalgae biomass. Green tides are of growing global concern due to their substantial ecological and economic impacts (Smetacek and Zingone, 2013); for example, the cost of maintaining an algae-free sea area near Qingdao for the 2008 Beijing Olympics sailing competition exceeded US$100 million (Wang et al., 2009). Curiously, *Ulva* is the dominant genus contributing the majority of green tide events (Fletcher, 1996).

The environmental changes caused by human activities would pose an effect on the physiological and biochemical traits of *Ulva*, an ecologically and economically important genus. However, little has been studied on the physiological traits and chemical composition of *Ulva* in the

* Corresponding author.
E-mail address: gary.caldwell@ncl.ac.uk (G.S. Caldwell).
[1] Present address for Guang Gao: Marine Resources Development Institute of Jiangsu, Lianyungang, 222001, China.

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context of the effects of ocean acidification, warming and eutrophication but some indications can be obtained from the effects of higher CO_2, temperature and nitrate levels.

Higher temperatures can usually stimulate the physiological performances of *Ulva*. For instance, the number of settled zoospores in *U. intestinalis* increased with temperature with the maximum at 23 °C (Christie and Shaw, 1968). Likewise, the bound zoospores of *U. compressa* increased from ~150 cells mm^{-2} to ~450 cells mm^{-2} when the temperature rose from 5 °C to 25 °C (Callow et al., 1997). The germination rate of *U. fasciata* was also enhanced by higher temperature, with the highest germination rate (78.53 ± 10.05%) at 25 °C (Mantri et al., 2011). In terms of growth and reproduction, the growth rate of *U. fenestrata*, collected from 6 °C seawater in Japan, was 3.349 ± 0.398% at 5 °C and 40 μmol photons m^{-2} s^{-1} while it was 6.559 ± 0.312% at 10 °C and 40 μmol photons m^{-2} s^{-1} (Kalita and Tytlianov, 2003). The reproduction rate of *U. fenestrata* increased from 6.1 ± 3.6% to 71.3 ± 31.8% when the temperature was increased from 10 to 15 °C (Kalita and Tytlianov, 2003). Regarding biochemical composition, the content of sugars and amino acids in *U. fasciata* increased with the rise of temperature (from 15 to 25 °C), reaching their maximum around 25 °C (Mohsen et al., 1973). The high temperature of 25 °C decreased the total lipid of *U. pertusa* from 2.7–3.6% dry weight to 2.6–2.7% dry weight compared to the low temperature of 15 °C (Floreto et al., 1993).

As for most organisms studied from an ocean acidification context, the experimental outcomes vary and appear to be species-dependent. For instance, growth of *Porphyra yezoensis* juveniles increased with CO_2 (350 to 1600 ppm) (Gao et al., 1991) as did growth of *U. prolifera* (1000 ppm) following an acclimation period (Xu and Gao, 2012). On the other hand, negative effects were observed on photosynthesis in *Ulva* spp. (Bjork et al., 1993), as well as growth in *Gracilaria tenuistipitata* (García-Sánchez et al., 1994), *P. leucostica* (Mercado et al., 1999), *P. linearis* (Israel et al., 1999) and *Fucus vesiculosus* (Gutow et al., 2014). In addition, recent studies have demonstrated that *U. rigida* (Rautenberger et al., 2015) and the giant kelp *Macrocystis pyrifera* (Fernández et al., 2015) are insensitive to ocean acidification (~1220 μatm pCO_2). Effects of high CO_2 levels on the settlement, germination and reproduction of *Ulva* have not yet been studied. In terms of biochemical composition, high CO_2 concentration (10,000 ppm) did not significantly affect total internal carbon, nitrogen or soluble carbohydrate in *U. rigida* but reduced soluble protein compared with the normal CO_2 level (350 ppm, Gordillo et al., 2001a, 2001b).

Nitrate is one of most important factors affecting *Ulva* growth. Research by Steffensen (1976) demonstrated that the addition of nitrate stimulated growth of *U. lactuca* with optimum levels being 43 μmol L^{-1}. The specific growth rate of *U. rigida* is also positively related to dissolved inorganic nitrogen (DIN) in the water column when DIN varies from 3 to 75 μmol L^{-1} (Viaroli et al., 1996). The only literature reporting nutrient effects on *Ulva* reproduction is from Mohsen et al. (1974). Their research demonstrated that nitrogen enrichment induced rapid sporogenesis and sporulation, whereas depleted nitrogen led to zygospore formation. Higher nitrate levels commonly stimulate the synthesis of amino acids and then protein content of *Ulva* (Naldi and Wheeler, 1999; Msuya and Neori, 2008; Angell et al., 2014). For instance, the total amino acid content of *U. ohnoi* increased linearly with internal nitrogen content ($r = 0.987$) with a range from 2.98 g 100 g^{-1} dry weight to 18.72 g 100 g^{-1} dry weight (Angell et al., 2014). Nitrogen concentration in the culture medium can regulate the degree of cellular lipid accumulation (Brennan and Owende, 2010). Nitrogen limitation enhanced total lipid of *U. rigida* from 64 mg g^{-1} dry weight to 72 mg g^{-1} dry weight at ambient CO_2 concentration (350 ppm) (Gordillo et al., 2001a). No reports on high nitrate levels affecting settlement and germination of *Ulva* have been found.

The findings of previous studies are helpful in understanding how ocean acidification, warming, or eutrophication alone affects the physiological or biochemical traits of seaweeds. However, to the best of our knowledge, none of the previous studies have examined the outcomes of the interactive effects of multiple climate change variables on life history and biochemical traits of *Ulva*. Neither ocean warming nor acidification are proceeding in isolation, rather there are also concurrent changes in nutrient levels. Given the ecological and socio-economic impacts of *Ulva* green tides we examined the interactive effects of ocean warming, acidification, and eutrophication on a selection of life history (gamete settlement, germination, growth, and reproduction) and key biochemical traits of *U. rigida*, a major green tide species (Fletcher, 1996). This research was undertaken with a view to predicting the future responses of green tides to ongoing global climate change.

2. Materials and methods

2.1. Sample identification, preparation and culture conditions

Ulva plants of 50–60 mm in length were collected from the low intertidal zone of Cullercoats Bay, Tyne and Wear, UK (55.03°N, 1.43°W) after a spring tide in May 2014. The fronds were placed in zip-lock plastic bags and transported to the laboratory within 1 h where they were gently rinsed in filtered (1 μm) natural seawater to remove any sediment, epiphytes and small grazers. The *Ulva* species used in this study was identified by DNA barcoding at the Institute of Oceanology, Chinese Academy of Sciences. It was found that the sequence, excluding the primers at both ends, fully matched (100%) to *U. rigida* SSBO0102 isolated from Skara Brae, Orkney, Scotland (Gao, 2016).

To determine whether life stage affects responses to the experimental factors assayed here, both adults and gametes of *U. rigida* were used. Seven hundred and twenty adult vegetative *U. rigida* fronds of 50–60 mm in length were haphazardly assigned to 24 identical Perspex tanks, each containing 10 L of natural seawater. Natural seawater was collected from the Blue Reef Aquarium®, Tynemouth, Tyne and Wear, UK (55.03°N, 1.43°W), very close to the *U. rigida* collection site. Gametes were obtained from fertile plants collected during a spring tide in June 2014 and treated as above. Gametes were released into suspension after exposing the fronds to light (fluorescent tubes, 80 μmol photons m^{-2} s^{-1}) for 1–2 h. The gamete suspension was transferred to a 500 mL beaker and the cells were concentrated by phototaxis to a point source light. They were then collected by pipette and transferred to filtered seawater. This step, which selected for healthy gametes and excluded most non-phototactic organisms, was repeated three times. Gametes were checked microscopically for the presence of two flagella and were positively identified as gametes given their positive phototaxis. Afterwards, collected gametes were used for settlement, germination and growth experiments.

The interactive effects of ocean acidification and warming under nitrate-limited and replete conditions were investigated using a fully crossed factorial design. The mature plants and gametes were cultured separately under the same treatment conditions in combinations of two pH (7.95, 7.55; coded as low carbon, LC and high carbon, HC respectively), temperature (14, 18 °C; coded as low temperature, LT and high temperature, HT respectively) and nitrate (6, 150 μmol L^{-1}; coded as low nitrate, LN and high nitrate, HN respectively) levels. The phosphate concentration was arbitrarily set at 50 μmol L^{-1} to obviate phosphorus limitation. Three replicate tanks were run for each treatment. Temperature was controlled using laboratory incubators with a photoperiod of 16 h light:8 h dark. Light intensity was 80 μmol photons m^{-2} s^{-1}. The ambient pH (7.95), nitrate concentration (6 μmol L^{-1}) and summer average surface seawater temperature (14 °C) of the North Sea (Mathis et al., 2015) were set as the control conditions. The reduced pH and elevated temperatures used represent the predicted levels by the year 2100 (Baede et al., 2001). The available nitrate concentration was maintained daily by addition of $NaNO_3$ following measurement by a rapid spectrophotometer method (Collos et al., 1999). Seawater was renewed every three days.

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